ENERGY TRANSDUCTION IN PHOTOSYNTHETIC BACTERIA
III. Coincidence of coupling factor of photosynthesis and respiration in *Rhodopseudomonas capsulata*

A. BACCARINI-MELANDRI and B. A. MELANDRI
Institute of Botany, University of Bologna, 40126 Bologna, Italy

Received 24 January 1972

1. Introduction

Recent work in our laboratory has dealt with the enzyme catalyzing ATP synthesis in membranes of facultative photosynthetic bacteria (specifically *Rhodopseudomonas capsulata*). The aim pursued by these studies, beyond the mere extension of previous researches on coupling factor proteins to prokaryotic photosynthetic organisms, was a comparison of the mechanism of photosynthetic and oxidative phosphorylation. In fact non-sulfur purple bacteria offer a very suitable model system for this purpose, since membrane preparations from these microorganisms can carry out either cyclic photophosphorylation or oxidative phosphorylation at rates depending on the conditions of growth (i.e. photosynthetic or aerobic).

Previously we reported about the reversible resolution of the photophosphorylating system [1], the purification and properties of a coupling factor protein from photosynthetic membranes [2] and its involvement in energy conservation [3]. Subsequently we have described a similar resolution of the respiratory system and the successful restoration of oxidative phosphorylation by coupling factor prepared from photosynthetic cells [4]. These results led us to suggest that in non-sulfur purple bacteria functional interchangeability between coupling factors of photosynthesis and respiration exists. These conclusions were also supported [4] by the demonstration that an antibody against photosynthetic coupling factor could inhibit indifferently photophosphorylation in pigmented membranes and oxidative phosphorylation in aerobic membranes. In this paper we present definite evidence that a coupling factor protein can be isolated from aerobically grown cells, which, after partial purification, is indistinguishable from the one obtained from photosynthetic cells. This protein can restore ATP synthesis and ATPase in both systems with an action that does not involve only structural reintegration of uncoupled membranes. A brief account of these studies has been presented at the IInd International Congress on Photosynthesis Research, Stresa, 1971.

2. Results and discussion

When *Rps. capsulata* is grown in the dark under strong aeration, the pigment content per cell is repressed very markedly and respiration ability is enhanced. These regulatory phenomena are, however, never so severe to suppress completely one of the two functions (several studies on this topic can be found in the literature, see for example [6–8] and also [5]). The procedure previously described [2] for the purification of the photosynthetic coupling factor proved to be successful also when membranes prepared from bacteria grown aerobically were used as starting material. Comparable yields during the purification steps, identical stability properties and coincident elution volumes during Sepharose 6B filtration [5], were indicative of the similar nature of the active factor in the 2 preparations. The electrophoretic patterns of the purest fractions of coupling factor prepared from photosynthetic or aerobic cells (and indicated as PCF and ACF, respectively) are shown in

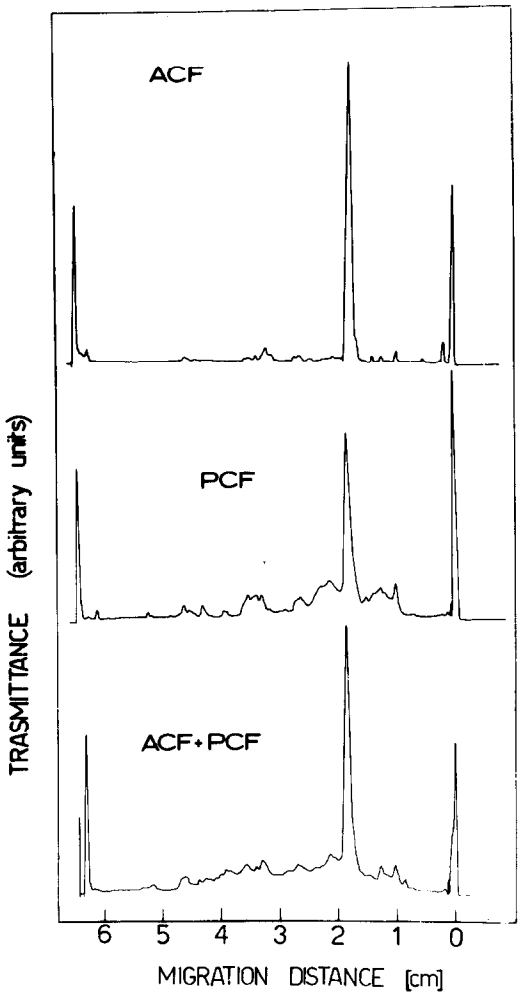

Fig. 1. Electrophoretic pattern of coupling factors from aerobic (ACF) or photosynthetic (PCF) membranes. The lyophilized samples were dissolved in phenol–acetic acid–urea and electrophoresed on acrylamide for 2 hr at 3 mA per tube as described by Takayama and Stoner [13]. The densitometric traces of the gels, stained with amido black, were recorded at 620 nm using a Chromoscan apparatus (Joyce and Loebl) equipped with the 5-077 C amplification triangle.

fig. 1. Although the purity of the protein is not complete, as is apparent from the minor contaminating bands, no difference could be detected in the mobility of the predominant component of the 2 preparations, even in co-electrophoresis.

Aerobic coupling factor restores with comparative efficiency oxidative phosphorylation in aerobic particles and photophosphorylation in photosynthetic particles (fig. 2). The saturation level obtained with either one of the 2 factors is identical; moreover, the maximum rate reached with one factor is not further stimulated by addition of the second [5].

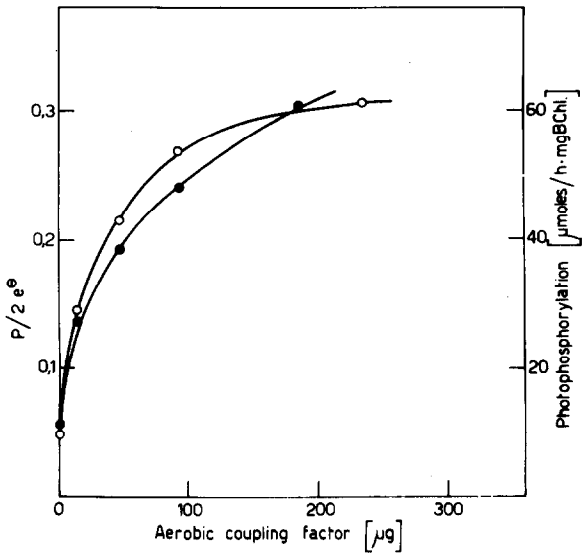

Fig. 2. Effect of purified aerobic coupling factor on photophosphorylation and NADH-dependent oxidative phosphorylation. Uncoupled aerobic particles (280 μg protein) or uncoupled photosynthetic particles (33 μg BChl.) were incubated for 20 min at 30° with variable amounts of purified ACF. The preparation of the two kinds of uncoupled membranes was carried out as described [4, 1]. Measurements of light-induced phosphorylation and NADH-dependent phosphorylation were performed at pH 8.0 and pH 7.2, respectively, following the procedures described in [2 and 4]. NADH oxidase was measured spectroscopically at 30° in a reaction mixture containing: 100 mM glycylglycine buffer pH 7.2; 10 mM $MgCl_2$; 1 mM EDTA; 2 mg/ml BSA and variable amount of particles. The reaction was started by addition of 0.15 mM NADH.

The complete reconstitution of the phosphorylating system by ACF, or indifferently by PCF, is confirmed also by its ability in restoring Mg^{2+} dependent, oligomycin sensitive ATPase in aerobic membranes (table 1). Similar results can be obtained using photosynthetic resolved membranes.

Schatz et al. [9] reported that yeast F_1 could stimulate oxidative phosphorylation in beef heart submitochondrial particles; however, in their system an antibody against yeast F_1 did not inhibit beef

Table 1
Reconstitution of Mg^{2+} dependent ATPase activity in uncoupled aerobic membranes.

Additions	ATPase activity (μ moles P_i hydrolysed/hr/mg prot.)
none	0.092
ACF (24 μg)	3.18
ACF (77 μg)	5.93
ACF (123 μg)	6.04
none	1.33
PCF (25 μg)	4.45
PCF (50 μg)	8.20
PCF (50 μg) + oligomycin (5 μg)	3.42

The procedure of recoupling was identical to that described in fig. 2. Oligomycin was added at the end of the incubation time. ATPase activity was measured at 30° at pH 8 (see [2].) The inorganic phosphate released by hydrolysis of ATP was measured according to Taussky and Shorr [13].

heart F_1 ATPase and was also unable to inhibit yeast F_1 stimulated phosphorylation in beef submitochondrial particles. This lack of effect was taken as evidence that the stimulation by the heterologous coupling factor was due merely to its structural effect on the membrane integrity. The possibility that also in this bacterial system similar structural effects may be involved, should be considered. An outstanding difference between *Rps. capsulata* system and that described by Schatz et al., was found by using a similar immunological approach: an antibody prepared against PCF can, in fact, inhibit both photosynthetic [10] or aerobic phosphorylation with the same efficiency [4].

Additional evidence against this possibility was obtained by an indirect approach. It is now well documented that oligomycin, at very low concentrations, can stimulate oxidative phosphorylation, and other energy dependent reactions, in submitochondrial particles partially depleted of F_1 [11, 12]. The interpretation is that oligomycin can simulate the structural role of coupling factors preventing the fast breakdown of a high energy intermediate. Oligomycin has, indeed, a limited stimulating effect on the $P/2e^-$ ratio, when tested on resolved membranes prepared from aerobic bacterial cells, at concentrations lower than 1 μg/mg of protein (fig. 3). On the contrary, if the particles are recoupled indifferently with an aerobic or photosynthetic coupling factor, the antibiotic is found to be always inhibitory, even at very low concentrations. In this respect recoupled membranes

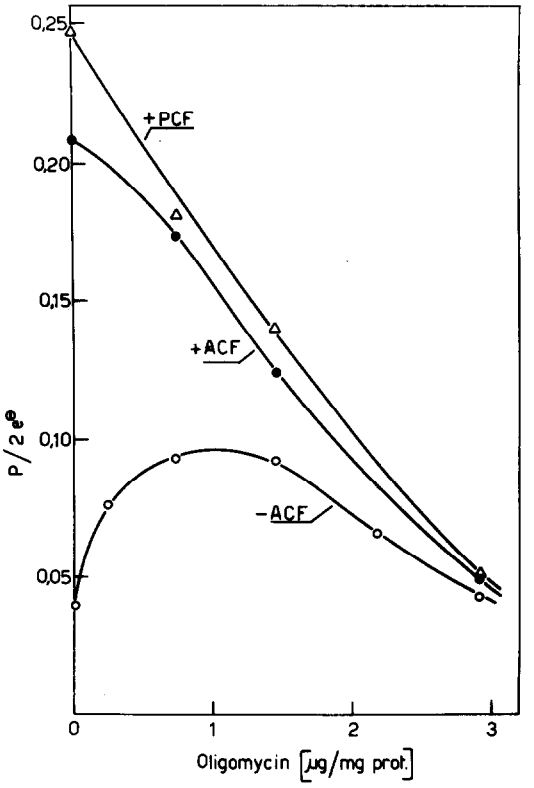

Fig. 3. Effect of oligomycin and coupling factors on NADH-dependent oxidative phosphorylation of resolved aerobic membranes. Uncoupled aerobic membranes (660 μg protein) were incubated at 30° for 20 min, in the absence of recoupling proteins or in the presence of saturating amounts of ACF or PCF, in a reaction mixture containing all the components except $^{32}P_i$ and NADH. At the end of the incubation period, oligomycin dissolved in a small volume of ethanol was added. The reaction was started by addition of 10 mM $^{32}P_i$ and 4 mM NADH and was carried out for 3 min.

behave exactly like the native coupled preparations. These data are interpreted as proof that in recoupled membranes all molecules of oligomycin interact with phosphorylation sites that are catalytically active.

All the experiments described here are consistent with the concept that in *Rps. capsulata* (and probably in other non-sulfur purple bacteria) the same coupling factor catalyzes both oxidative and photosynthetic phosphorylation. This conclusion is supported by identical characteristics of ACF and PCF during purification and storage, by coincident mobility in disc gel electrophoresis and by similar and non-additive functional action [5]. Both preparations appear to contain an enzyme that can stimulate phosphorylation being actually involved in a catalytic step of the energy transduction process. This coupling factor, present in a facultative photosynthetic bacterium, represents therefore the first example, so far described, of a protein involved indifferently in ATP generation dependent on respiration or photosynthesis. This conclusion agrees with the dual role often postulated for energy transducing membranes in Athiorhodaceae.

References

[1] A. Baccarini-Melandri, H. Gest and A. San Pietro, J. Biol. Chem. 245 (1970) 1224.

[2] A. Baccarini-Melandri and B. A. Melandri, in: Methods in Enzymology, Vol. 23, ed. A. San Pietro (Academic Press, New York, 1971) p. 556.

[3] B. A. Melandri, A. Baccarini-Melandri, A. San Pietro and H. Gest, Proc. Natl. Acad. Sci. U.S. 67 (1970) 477.

[4] B. A. Melandri, A. Baccarini-Melandri, A. San Pietro and H. Gest, Science 174 (1971) 514.

[5] A. Baccarini-Melandri and B. A. Melandri, in: Proceedings of IInd International Congress on Photosynthesis Research, Stresa, 1971.

[6] D. L. Keister, in: Progress in Photosynthesis Research, Vol. III, ed. H. Metzner (Tubingen, 1969).

[7] A. Thore, D. L. Keister and A. San Pietro, Arch. Mikrobiol. 67 (1969) 378.

[8] F. D. Whale and O. T. G. Jones, Biochem. Biophys. Acta 223 (1970) 157.

[9] G. Shatz, H. S. Penefsky and E. Racker, J. Biol. Chem. 242 (1967) 2552.

[10] B. A. Melandri and A. Baccarini-Melandri, in: Proceedings of IInd International Congress on Photosynthesis Research, Stresa, 1971.

[11] C. P. Lee and L. Ernster, Biochem. Biophys. Res. Commun. 18 (1965) 523.

[12] J. M. Fessenden and E. Racker, J. Biol. Chem. 241 (1966) 2483.

[13] K. Takayama and C. D. Stoner, in: Methods in Enzymology, Vol. X, eds. R.W. Estabrook and M.E. Pullman (Academic Press, New York, London) p. 676.

[14] H. Taussky and E. Shorr, J. Biol. Chem. 202 (1953) 675.